Claims

What is claimed is:

1. A phosphoprotein detection reagent (PPDR) comprising a chelator-metal ion moiety and an detectable moiety conjugated to the chelator-metal ion moiety, wherein the chelator-metal ion moiety selectively binds to a phosphorylated amino acid residue in a phosphoprotein if present to create a chelator-metal ion-phosphoprotein (CMPP) complex, and the detectable moiety allows the CMPP complex to be detected if present.

2. The PPDR of claim 1, wherein the PPDR is soluble in an aqueous medium.

3. The reagent of claim 1, wherein the chelator is nitriloacetic acid.

4. The reagent of claim 1, wherein the chelator is iminodiacetic acid.

5. The reagent of claim 1, wherein the metal ion is chosen from the group consisting of Fe^{3+}, Cu^{2+}, Al^{3+}, Yb^{3+}, Zn^{2+}, Ni^{2+}, Co^{2+}, and Ga^{3+}.

6. The reagent of claim 5, wherein the metal ion is Ga^{3+}.

7. The reagent of claim 5, wherein the metal ion is Fe^{3+}.

8. The reagent of claim 1, wherein the detectable moiety is biotin.

9. The reagent of claim 1, further comprising a spacer between the chelator-metal ion moiety and the detectable moiety.

10. A method for synthesizing a PPDR that is soluble in an aqueous medium, the method comprising:

(a) reacting a polydentate chelator donor molecule with a detectable moiety donor under conditions wherein a detectable moiety is

transferred to a polydentate chelator to form a chelator-detectable moiety complex; and

(b) mixing the chelator-detectable moiety complex and a metal ion-containing solution under conditions wherein the chelator-detectable moiety complex coordinates the metal ion, forming a PPDR that is soluble in aqueous medium.

11. The method of claim 10, wherein the chelator donor molecule is selected from the group consisting of 2-(aminooxyethyl)iminodiacetic acid (AIDA), aminobutyl-nitriloacetic acid (AB-NTA), and iminodiacetic acid (IDA).

12. The method of claim 10, wherein the detectable moiety donor is selected from the group consisting of sulfo-N-hydroxysuccinimidyl-biotin (sulfo-NHS-biotin), sulfosuccinimidyl-6-(biotinamido) hexanoate (sulfo-NHS-LC-biotin), sulfosuccinimidyl-6-(biotinamido)-6-hexanimido hexanoate (sulfo-NHS-LC-LC-biotin), and penta-fluorophenyl-biotin.

13. The method of claim 10, wherein the detectable moiety donor is present in the reacting step in a molar excess over the polydentate chelator donor molecule.

14. The method of claim 10, wherein the chelator-detectable moiety complex and a metal ion-containing solution are present in equimolar concentrations in the mixing step.

15. A method for detecting a phosphoprotein, the method comprising:

(a) obtaining a protein-containing solution;

(b) separating the proteins present in the solution from each other;

(c) contacting the proteins with a reagent under conditions wherein the reagent will selectively bind to a phosphorylated amino acid residue present within the proteins to form a reagent/amino acid complex, the reagent comprising a chelator-metal ion moiety

and an detectable moiety conjugated to the chelator-metal ion moiety, wherein the chelator-metal ion moiety selectively binds to a phosphorylated amino acid residue in a phosphoprotein if present to create a chelator-metal ion-phosphoprotein (CMPP) complex, and the detectable moiety allows the CMPP complex to be detected if present; and

(d) detecting the reagent/amino acid complex, wherein the detection of the reagent/amino acid complex detects a phosphoprotein.

16. The method of claim 15, wherein the separating is by electrophoresis.

17. The method of claim 16, wherein the separating is by two-dimensional gel electrophoresis.

18. The method of claim 15, wherein the separating is by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

19. The method of claim 15, wherein the conditions wherein the reagent will selectively bind to a phosphorylated amino acid residue present within the immobilized proteins to form a reagent/amino acid complex comprise permissive conditions followed by washing the solid support to remove unbound reagent.

20. The method of claim 19, wherein the permissive conditions comprise contacting the reagent and immobilized proteins at a pH between about 5.0 and 7.0, and washing at a pH between about 6.9 and 9.5.

21. The method of claim 15, wherein the detecting is via a chemiluminescent assay.

22. The method of claim 15, wherein the detecting is via fluorescence.

23. The method of claim 15, wherein the detecting is via a colorimetric assay.

24. The method of claim 15, further comprising the step of immobilizing the proteins on a solid support after the separating step and prior to the contacting step.

25. The method of claim 24, wherein the immobilizing is by electrophoretic transfer.

26. The method of claim 24, wherein the solid support is a PVDF membrane.

27. The method of claim 15, further comprising the step of treating the proteins with a carboxy-blocking reagent after the separating step and prior to the contacting step.

28. The method of claim 27, wherein the carboxy-blocking reagent is selected from the group consisting of methanolic HCl, a carbodiimide, and Woodward's Reagent "K".

29. A method for detecting a change in phosphorylation status of a protein present within a target tissue in response to a change in state, the method comprising:

(a) obtaining a protein lysate from a cell from the target tissue prior to the change in state;

(b) separating the proteins present in the lysate from each other;

(c) contacting the proteins with a reagent under conditions wherein the reagent will selectively bind to a phosphorylated amino acid residue present within the proteins to form a reagent/amino acid complex, the reagent comprising a chelator-metal ion moiety and an detectable moiety conjugated to the chelator-metal ion

moiety, wherein the chelator-metal ion moiety selectively binds to a phosphorylated amino acid residue in a phosphoprotein if present to create a chelator-metal ion-phosphoprotein (CMPP) complex, and the detectable moiety allows the CMPP complex to be detected if present;

(d) detecting the reagent/amino acid complex, wherein the reagent/amino acid complex is indicative of a phosphoprotein in the cell lysate;

(e) creating a profile indicative of the detected phosphoproteins of the lysate;

(f) obtaining a protein lysate from a cell from the target tissue after the change in state;

(g) repeating steps b) through e) for the lysate from a cell from the target tissue after the change in state; and

(h) comparing the profile from the lysate from the cell from the target tissue prior to the change in state to the profile from the lysate from the cell from the target tissue after the change in state, wherein a difference between the two profiles is indicative of a change in the phosphorylation status of a protein present within the target tissue in response to the change in state of the target tissue.

30. The method of claim 29, wherein the change in state is from a non-neoplastic to a neoplastic state.

31. The method of claim 29, wherein the change in state is from a non-differentiated to a differentiated state.

32. The method of claim 29, wherein the change in state is from a benign state to a malignant state.

33. The method of claim 29, wherein the change in state is from an unstimulated to a stimulated state.

34. The method of claim 29, further comprising the step of immobilizing the proteins present in the solution onto a solid support prior to the detecting step.

35. A method for early diagnosis of a change in state of a target tissue, the method comprising:

(a) detecting a phosphorylation state of a protein in a target tissue and

(b) comparing the detected phosphorylation state of the protein to a standard profile, wherein the comparison identifies a change in state of the target tissue.

36. A kit comprising the PPDR of claim 1.

37. The kit of claim 36, further comprising instructions for using the PPDR.

38. The kit of claim 36, further comprising a secondary reagent for detecting the PPDR.